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A REVIEW ON BIOLOGICAL CONTROL OF SOME IMPORTANT PLANT DISEASES USING FUNGAL ANTAGONIST, TALAROMYCES FLAVUS

LALEH NARAGHI & ASGHAR HEYDAR

Iranian Research Institute of Plant Protection, Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran

ABSTRACT

Talaromyces flavus is an important antagonist fungus that plays an important role in the prevention of important pathogenic herbaceous plant diseases. This fungus is located in the rhizosphere of plants, uses root permeations, and produces hydrogen peroxide with the help of glucose oxidase enzymes in the presence of glucose in these compounds. Hydrogen peroxide is a highly toxic compound for fungi and pathogenic bacteria, which can destroy them. The inhibitory mechanisms of this fungus for the growth of pathogenic agents include microparasiticity by chitinase and pectinase enzymes, production of volatile compounds of alcoholic and aldehyde, and the production of non-volatile compounds by the glucose oxidase enzymes, galactosidase and giziosidase. In Iran, the efficiency of various T. flavus isolates has been investigated in the prevention of important pathogens and, consequently, the control of related diseases. The results showed that the T. flavus isolates from different soil and crops, such as cotton, potatoes, sugar, beet, tomatoes and greenhouse cucumbers, each had the most impact on the control of the related diseases. Also, the incidence of the diseases studied (cotton verticillom, potato verticillom, sugar beet seedling death, tomato fusarium wilting and greenhouse cucumber fusarium wilting) decreased by about 30%. Apart from playing an effective role in controlling disease, T. flavus also has a role in the development of vegetative traits like height, dry weight, fresh weight and yield of these products. The plant growth enhancement properties are the result of the production of similar compounds with growth phytohormones of auxin, cytokinin and gibberellin, and fungal intervention in the synthesis of phytohormones by the plant. For application of this fungus in the greenhouse and in the field, its mass production was carried out on various vegetable substrates, including wheat bran, rice bran, pit soil, wheat shrimp, corn wood corn, and pit soil, mixed with rice bran. The relevant research has shown that the most suitable bed was the rice bran bed in terms of efficiency in increasing the active population of fungi for isolates of cotton potato and sugar beet. However, the highest active population of the fungus was observed for tomato and greenhouse cucumber isolates on the pit soil with rice bran. In other studies in order to increase the stability of the fungus on the propagation substrates the stabilizers for volatile and non-volatile compounds, including aminophenol, arboxymethyl cellulose, dicycloserine, sodium nitrate, and magnesium sulfate were used. The results showed that the highest increase in the active population of the fungus occurred after one year of its mass production, with the use of stabilizers of sodium nitrate and dicycloserine.

KEYWORDS: Biological Control, Cotton, Greenhouse Cucumber, Plant Fungal Disease, Potato, Sugar Beet, Talaromyces Flavus & Tomato

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Original Article

INTRODUCTION

Pests (harmful insects, parasitic weeds, and pathogens) are among the most important biotic agents causing

serious losses and damages to agricultural products. Plant pests need to be controlled to ensure the food, feed, and fiber production quantitatively and qualitatively. A number of different strategies are currently being employed to manage and control plant pests. Beyond good agronomic and cultural practices, growers often rely heavily on the chemical pesticides application. However, the environmental contamination caused by excessive use of Agrochemicals has led to considerable changes in people's attitudes towards the use of pesticides in agriculture. Today, there are strict regulations on chemical pesticide use, and there is political pressure to remove the most hazardous chemicals from the market. In addition to the above-mentioned issues, the spread of plant diseases in natural ecosystems may preclude successful application of chemicals, because of the scale to which such applications might have to be applied. Consequently, some pest management researchers have focused their efforts on developing alternative inputs to synthetic chemicals for controlling pests and diseases.

Verticillum and Fusarium wilts are serious diseases on many crops, including cotton, greenhouse cucumber, potato and tomato causing considerable quantitative and qualitative losses in their production. Due to the soil-born nature of the disease, application of chemical fungicides has not shown efficacy in controlling this disease. The use of resistant varieties which is the most common strategy for combating this disease is very costly and time-consuming. Biological approaches using microbial antagonists have been considered a viable method for the management of Verticillium wilt disease and its causal agent *Verticillium dahliae.* Among various microbial antagonists, *Talaromyces flavus,* a fungal antagonist have shown to be a novel and effective biofungicide in this regard. Cotton is an important cash crop around the world grown in more than 80 countries including Iran. Cotton is cultivated in about 20 provinces of Iran (Heydari *et al*., 2005; Ardekani *et al*., 2009; Shahraki *et al*., 2009; Heydari and Pessarakli, 2010; Naraghi *et al*., 2010a, b and c; Naraghi *et al*., 2011; Kakvan *et al*., 2013; Mansoori *et al*., 2013).

Harmful pests (insects, weeds and pathogens) are among the most important yield reducing agents in cotton fields (Baston and Blasengame, 1988; Bendict *et al*., 1989; Heydari *et al*., 2005; Heydari and Misaghi, 1998; Naraghi *et al*., 2007; Watkins, 1981). Verticillium wilt and Rhizoctonia seedling damping- off are considered the most important diseases of cotton in Iran (Heydari *et al.,* 2007; Naraghi *et al.*,2007). For controlling these diseases, cultural practices and the use of resistant varieties are the most common strategies which are not either available or effective. Biological control using fungal and bacterial antagonists in recent years, have been applied to control cotton diseases (Heydari, *et al.* 1997; Heydari & Misaghi, 1998; Heydari & Misaghi, 2003; Heydari *et al*.,2007; Spinks & Rowe, 1989; Aziz, *et al*., 1997).

Talaromyces flavus (Klocker) Stulk & Samson is a fungal antagonist that has been used in biological control of some important plant diseases (Marois, *et al*., 1984; Tjamos & Fravel, 1995; Naraghi, *et al*., 2004). Marois *et al.* (1982) showed that *T. flavus* decreased the incidence of Verticillium wilt and increased yield of egg-plant in England. Fahima and Henis (1997) showed that *T. flavus* decreased Verticillium wilt disease on egg-plant at the rate of 77%. The ability of this fungus for occupying rhizosphere of cotton, egg-plant and decreasing germination of microsclerotia of *Verticillium dahliae* have been reported (Marois, *et al.*, 1984). *T. flavus* and *Aspergillus terreus* have also been reported to be the inhibitory agents of Verticillium wilt of olive trees in Greece (Tjamos, 1991). Forty percent of non-volatile extracts (Talaron) of *T. flavus* belongs to Glucose oxidase enzyme and the production of Hydrogen peroxide by this enzyme gives antibacterial and antifungal characteristics to this fungus (Kim & Fravel, 1990).

Greenhouse cucumber (*Cucumis sativus* L.) is another major crop and is widely grown in many parts of the world. The cultivation of vegetables in greenhouses is an expanding business world-wide. About 83 countries grow greenhouse

vegetables commercially, including greenhouse cucumber, tomato and paper commercially, totaling over 400,000 hectares. The average yield of greenhouse cucumber is 60 tons per hectare (Jilani *et al.*, 2009). In Iran, about 2300 hectares are under the cultivation of this crop, with an average yield of 200 tons per hectare (Soleimani *et al.*, 2009). Verticillium and Fusarium wilts are the most important diseases of greenhouse cucumber and cause serious losses in the field (Al-Rawahi *et al.*, 1998; Sanei *et al.*, 2008). *Verticillium dahliae* Kleb., *V. albo-atrum* Reinke & Berthold and *Fusarium oxysporum* f. sp. *cucumerinum* (Owen) Snyder and Hansen are the causal agents of this disease (Correll, 1988; Mansoori and Smith, 2005; Jabnoun-Khiareddine *et al.*, 2006; Roustaee and Baghdadi, 2007; Najafiniya and Sharma, 2011). For controlling Verticillium and Fusarium wilts of greenhouse cucumber, appropriate cultural practices and the use of resistant varieties are the most common strategies, but they are not always available or effective (Rekanovic *et al.*, 2007). Biological control using fungal and bacterial antagonists has been applied in recent years to manage greenhouse cucumber diseases (Singh *et al.*, 1999; Martin and Bull, 2002; Naraghi *et al.*, 2006 ; Naraghi *et al.*, 2008; Heydari and Pessarakli, 2010; Naraghi *et al*., 2010a; Naraghi *et al*., 2017).

Talaromyces flavus (Klocker) Stulk and Samson is an antagonistic fungus that has been used for the biological control of some soil-borne pathogens such as *V. dahliae*, *V. albo-atrum*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum* (Marois *et al.*, 1984; Tjamos and Antoniou, 2000; Punja, 2001; Brunner *et al.*, 2005; Gohel *et al.*, 2006). Marois *et al.* (1984) also reported that this fungus grows on the rhizosphere of greenhouse cucumber. *Talaromyces flavus* (the teleomorph of *Penicillium dangeardii*) was also reported as a parasite of sclerotia of *Rhizoctonia solani* and *Sclerotinia sclerotiorum* (McLaren *et al.*, 1982). More than 300 million tons of potato (*Solanum tuberosum* L.) is produced worldwide in 22 million hectares of land. Potato is a major food source and ranks fifth after wheat, barley, rice and maize (Secor and Gudmestad, 1999). About 140,000 hectares are grown in Iran, with an average yield of 22 tons per hectare (Anonymous, 2004). Verticillium wilt is one of the most important diseases of potato that attacks this plant and reduces the quality and quantity of its yield the fields (Aminaee *et al*., 2006). *Verticillium dahliae* Kleb. and *Verticillium albo-atrum* Reinke and Berthold are the causal agents of this disease (Mansoori and Smith, 2005; Aminaee *et al*.,2006; Naraghi *et al*., 2010a; Naraghi *et al*., 2017).

For the management of Verticillium wilt of potato, cultural practices and the use of resistant varieties are the most common methods, but are not always available or effective (Thanassoulopoulos and Hooker, 1968). Biological control using fungal and bacterial antagonists in recent years, have been applied to control potato diseases (Schisler *et al*., 1996; Safari and Rouhani, 1998 ; Sadafi *et al*., 2001 ; Khorasani Aghazadeh *et al*.,2008). *Talaromyces flavus* is a fungal antagonist that has been used in biological control of some soil-borne pathogens such as *Verticillium dahliae*, *Verticillium albo-atrum*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum* (Marois *et al*., 1984). Marois *et al*.(1984) also reported that this fungus can occupy the rhizosphere of potato and decreased germination of the fungal pathogen.

Tomato (*Lycopersicon esculentum* Mill.) is another food crop which is the second most important vegetable crop next to the potato. The present world production is about 100 million tons of fresh fruit produced on 3.7 million hectares. Tomato production has been reported in 144 countries (Bal and Abak, 2007). Iran is ranked seventh in the world as a tomato producer. In Iran tomato is planted on about 140000 hectares with an average yield of 37 tons per hectare (Alemzadeh Ansari and Mamghani, 2008). Verticillium wilt is a serious disease in this crop causing considerable yield reduction annually (Paternotte and Van Kasteren, 1993 ; Matta and Garibaldi, 1997 ; Aminaee *et al.*, 2006). *Verticillium dahliae* and *Verticillium albo-atrum* are the causal agents of this disease (Pegg and young, 1982; Hutson and Smith, 1982;

Paternotte and Van Kasteren, 1993 ; Kim *et al*., 2001 ; Mansoori and Smith, 2005 ; Aminaee *et al.*, 2006). For controlling Verticillium wilt of tomato, cultural practices and the use of resistant cultivars are the most common strategies but they are either not available or not effective (Jones *et al*.,1995 ; Burbos and Skoudridakis, 1996 ; Giotis *et al*., 2009). In recent years, biological control using fungal and bacterial antagonists have been applied to control tomato diseases (Hanafi, 2003 ; El-Mougy *et al.*,2008 ; Giotis *et al*., 2009 ; Szpyrka and Sadllo, 2009 ; Oclarit and Cumagun, 2009).

The ability of *T. flavus* in occupying rhizosphere of tomato and decreasing germination of microsclerotia of *V. dahliae* has also been reported by Marois *et al*. (1984). Environmental concerns have led to the need for sustainable use of natural resources. The conventional agriculture has caused considerable negative impacts on soil and water. It is important to change certain management practices to environmentally cleaner techniques (Sudha Lakshmi *et al.*, 2011). The sustainable agriculture employs many approaches and techniques to reduce negative effects of conventional agricultural practices on the environment. One of these strategies is the utilization of soil microorganisms for the promotion of plant growth and control of plant diseases (Botelho and Hagler, 2006). The widespread use of chemical pesticides and fertilizers have been subject to public concerns and environmental protection agencies. The harmful chemicals may affect human health, contaminate the environment and negatively affects biological resources. In addition, their high production cost and appearance of resistant pests should also be considered (Zaki *et al*., 1998; Heydari and Misaghi, 1998 and 2003).

Some antagonistic fungi play direct and indirect roles in promoting plant growth characteristics. In a direct role, metabolites of antagonistic fungi promote plant growth by providing soluble elements which are necessary for plant nutrition. Indirectly, some microorganisms can act as biological control agents and affect plant growth promotion indirectly through decreasing plant diseases (Le Floch *et al.*, 2003). In addition to the antagonistic activities of *T. flavus*, it may have growth promotion ability due to the production of various metabolites and enzymes. Some of the above-mentioned metabolites (2-Methylsorbic acid, sorbic acid, bromomethylsorbic acid and bromosorbic acid) play a fundamental role in the biogeochemical cycling of phosphorous (P) in natural and agricultural ecosystems (Wakelin *et al.*, 2004).

Harvey *et al.* (2009) demonstrated that microbiological activity in the rhizosphere, could dissolve sparingly soluble inorganic phosphorous and increase plant growth. *T. flavus* are a common mycoflora member of cotton and potato. Since the Verticillium wilt disease is a major disease and causes serious damages to the above-mentioned crops, we studied the biological control of this disease using a novel biofungicide (*Talaromyces flavus*) in order to reduce the losses caused by this disease and protect the environment and natural resources. Results of our studies in this regard have been presented in chapters one to four. In addition, and due to the growth promotion ability of *T. flavus,* in the last part of the book, the results of our study on the impact of this fungus on the growth of cotton and potato have also been presented.

Biological Control of Verticillium Wilt Disease Using *Talaromyces Flavus*

Verticillium wilt is a wilt disease of over 350 plant species caused by six species of Verticillium genus, *V. dahliae*, *V. albo-atrum*, *V. longisporum*, *V. nubilum*, *V. theobromae* and *V. tricorpus* (Barbara and Clewes, 2003). *Verticillium* spp. attack a very large host range including more than 350 species of vegetables, fruit trees, flowers, field crops, and shade or forest trees. Most vegetable species have some susceptibility, so it has a very wide host range (Agrios, 2005). The symptoms are similar to most wilts with a few specifics to *Verticillium*. Wilt itself is the most common symptom, with wilting of the stem and leaves occurring due to the blockage of the xylem vascular tissues and therefore reduced water and nutrient flow. In small plants and seedlings, *Verticillium* can quickly kill the plant while in larger, more

developed plants the severity can vary. Sometimes only one side of the plant will appear infected because once in the vascular tissues, the disease migrates mostly upward and not as much radially in the stem. Other symptoms include stunting, chlorosis or yellowing of the leaves, necrosis or tissue death, and defoliation. Internal vascular tissue discoloration might be visible when the stem is cut. In *Verticillium*, the symptoms and effects will often only be on the lower or outer parts of plants or will be localized to only a few branches of a tree. In older plants, the infection can cause death, but often, especially with trees, the plant will be able to recover, or at least continue living with the infection. The severity of the infection plays a large role in how severe the symptoms are and how quickly they develop (Pegg and Brady, 2002; Agrios, 2005).

Talaromyces flavus (Klocker) Stulk and Samson is a fungal antagonist that has been used in biological control of Verticillium wilt of several plants such as cotton, potato, tomato and greenhouse cucumber (Marois *et al*., 1984; Tjamos and Fravel, 1995; Naraghi *et al*., 2004). Marois *et al*. (1982) showed that *T. flavus* decreased the incidence of Verticillium wilt and increased yield of egg-plant in England. Fahima and Henis (1997) showed that *T. flavus* decreased Verticillium wilt disease on egg-plant at the rate of 77%. The ability of this fungus for occupying rhizosphere of potato, cotton, egg-plant and decreasing germination of microsclerotia of *V. dahliae* have been reported by Marois *et al* (1984). Based on the previous research, it is concluded that the use of antagonistic fungi including *T. flavus* and its metabolites and ascospore suspension could be an effective method for controlling Verticillium wilt disease of cotton and may be considered a major component of integrated pest management (IPM) strategies for managing this disease which is a very important and destructive disease of cotton in all cotton growing areas in the world.

The results of the previous studies showed that it may be possible to manage cotton Verticillium wilt disease efficiently by seed treatment with non-volatile extracts or Ascospore suspension of *T. flavus* (Naraghi *et al*., 2008). The efficiency of non-volatile metabolites of some bacteria and fungi such as *Aspergillus flavus*, *A. ochraceus*, *Penicillium aurantiogriseum*, *Bacillus subtilis* and *Trichoderma harzianum* for controlling bean antracnose disease have been reported (Adebanjo and Bankole, 2004). Other examples, such as B-1,3 gluconase secreted by Burkholderia cepacia (Fridlender *et al*., 1993) and glucose oxidase produced by *T. flavus* (Murrary *et al*., 1997) have been effective for decreasing.

In another study, it has been shown that proxide, hydrogen prevented formation of microsclerotia of *V. dahliae* (Kim and Fravel, 1990). Since, the glucose oxidase enzyme produced by *T. flavus* acts in glucose presence and this activity is resulted in peroxide, hydrogen production, therefore, a glucose addition to non-volatile metabolite of T. flavus caused an increase in inhibitory effect on microsclerotia formation (Murrary *et al*., 1997).

Naraghi *et al*. (2014a) have also shown that it was possible to control Verticillium wilt disease caused by *V. dahliae* and *V. albo-atrum* using antagonist fungus (*T. flavus*) in potato fields. Based on the previous study (Naraghi *et al*., 2010a), there effective isolates of *T. flavus* were used which were initially isolated from potato fields in Varamin area. In the research related to Naraghi *et al*. (2014a) research, fungal antagonists, which were obtained from Varamin and Hamedan regions showed different efficacy which could probably be related to their growth temperature requirement and their responses to the potato root (rhizome) exudates. Concerning the above-mentioned, subjects, there have been several reports about optimal growth of *T. flavus* isolates within range of temperature 30-40°C and occupation of rhizosphere of some crops, including cotton, eggplant, potato, and tomato by fungus and the role of root exudations of these plants (Marois *et al*., 1984; Duo- Chuan *et al*., 2005).

Inceoglu *et al*. (2012) during their study about the impact of soil and plant cultivars on bacterial microorganisms on potato rhizosphere, reported that presence of glucose compounds in root exudations played important role in these interactions. Alternatively, antagonistic effects of *T. flavus* depend on glucose oxidase that its activity has been proved by the presence of glucose (Kim *et al*. 1990; Fravel & Robert, 1991). In the current study, in addition to isolation of potato verticillium wilt pathogenic agents (*V. dahliae* and *V. albo-atrum*), the population of their propagules number in the field soil as about 20 CFU/ gr was also calculated. In a previous study, the populations of *V. dahliae* and *V. albo-atrum* in potato fields in Hamedan, Tehran, Khorasan, Kerman, Kurdistan, Ardebil, and Isfahan provinces have been reported as about 33 CFU per gram of soil (Mansoori *et al*., 2006).

The field results of the previous studies showed that all treatments affected by antagonistic isolates of *T. harzianum* or *T. flavus* and Carbendazim fungicide reduced significantly in terms of the disease incidence and severity significantly and also increased the yield in comparison with the untreated control (Fravel, 1989; Bruner *et al*., 2005; Goicoechea, 2009; Narathi *et al*., 2014a).

Naraghi *et al*. (2014a) carried out the field experiments in two consecutive years. The results of the second year showed that there was no significant difference among the experimental treatments in terms of disease incidence and severity and yield. Comparison of the results in two crop years showed that in the first year, the significant difference in terms of disease and yield in two treatments of TF-Po-V- 49 and control played a remarkable role in the presence of a significant relationship between the tested treatments. In the second year, however, the disease and yield in both above-mentioned treatments with the reduction in disease (26.51 and 30.98%) and increase in yield (66.35 and 78.14%) approached to an average of these attributes in all treatments while they showed no significant difference from other treatments.

Therefore, In the conducted experiment in second year, it is concluded that due to the presence of the remaining potato rhizomes affected by *T. flavus* inoculum within the soil, employing of seed treatments by *T. flavus* led to an improvement in the population of these isolates in all treatments and lack of significant difference among them was measured in terms of all criteria. In similar studies, promotion of population of microorganisms in the soil of potato fields of application of affected treatments from antagonistic fungi has been reported (Lodhi, 2004; Berg *et al*., 2005).

The results of the study related to Naraghi *et al*. (2010c) showed that it might be possible to manage the tomato Verticillium wilt disease efficiently by seed treatment with effective *T. flavus* isolates. The inhibitory effect of volatile and non-volatile extracts of several bacterial and fungal microorganisms on growth and activity of some fungal pathogens was demonstrated in previous studies. For example, the volatile extracts of *F. oxysporum* resulted in induction of resistance to chickpea (*Cicer arietinum* L.) against fungal pathogens (Cherif *et al.* 2007). The efficiency of non-volatile extracts of some bacteria and fungi including *A. flavus*, *A. ochraceus*, *Penicillium aurantiogriseum*, *Bacillus subtilis* and *Trichoderma harzianum* for controlling antracnose of cowpea (*Vigna unguiculata* L. Walp.) were reported (Adebanjo and Bankole 2004).

In other studies, the effect of these extracts on different soil-borne fungal diseases of tomato was also shown (Josh *et al.* 2009). In another study, the effect of volatile and non-volatile extracts of *T. flavus* on root-rot disease of lettuce (*Lactuca sativa* L.) caused by *Sclerotinia minor* was shown in the laboratory and greenhouse experiments. Results of this study confirmed that the inhibitory effect of variable components of extracts on pathogenic agent growth was different (El-Tarabily *et al*. 2000). Results of another study on the use of non-volatile extracts such as chitinase produced by *T.*

harzianum and *T. flavus* were effective for controlling soybean stem white rot disease caused by *S. sclerotiorum* and bean stem rot caused by *S. Rolfsii*, respectively (Madi *et al*. 1997; Menendez and Godeas 1998). Other examples of fungal metabolites such as glucanase secreted by *Zygorrhynchus moelleri* and glucose oxidase produced by *T. flavus* have also been effective against some soil-borne plant pathogenic fungi (Brown 1987; Murrary *et al*. 1997).

Results of studies on the use of *T. flavus* in biocontrol of pathogenic fungi showed that *T. flavus* were an important antagonist on *V. dahliae* and *V. a.-a.* (Tjamos and Paplomatas 1987; Wikins *et al*. 2000; Vidhyasekaren 2004). Kim and Fravel (1990) showed that glucose oxidase produced by *T. flavus* prevented the formation of microsclerotia of *V. dahliae*. However, Proksa *et al*. (1992) showed that 2-methyl sorbic acid secreted by *T. flavus* showed an inhibitory effect on the growth of *V. a.-a.*

The differences between the results of the laboratory experiments in the present study and the previous ones could have been due to the extract concentration. It was reported that there was a minimum concentration (0.1 μg/ ml) for every effective component of non-volatile extracts of *F. oxysporum* (Cyclosporin) used for growth inhibition of *S. sclerotiorum* (Rodriguez *et al*. 2006). In another part of this study, using effective *T. flavus* isolates as a seed treatment decreased Verticillium wilt infection index in greenhouse conditions. These results agree with those of some previous studies (Soytong and Ratanacherdchai 2005; Kulikov *et al*. 2006). The minor differences between the results of this study and those of the previous ones could be due to a method of seed treatment with *T. flavus* isolates and to the intervals before sowing (Nagtzaam and Bollen, 1997).

Naraghi *et al*. (2010b) showed that Verticillium wilt of greenhouse cucumber is managed effectively by treating soil or seed with *T. flavus* isolates. Studies on using *T. flavus* for the biocontrol of pathogenic fungi have found that *T. flavus* is an important antagonist to *V. dahliae* and *V. albo-atrum* (Tjamos and Paplomatas, 1987; Wikins *et al.*, 2000; Vidhyasekaren, 2004). Kim and Fravel (1990) reported that glucose oxidase produced by *T. flavus* prevented the formation of microsclerotia of *V. dahliae*, and Proksa *et al.* (1992) showed that 2-methyl sorbic acid secreted by *T. flavus* had an inhibitory effect on *V. albo-atrum*.

Differences in the results obtained from the present laboratory experiments and those from previous experiments could be due to the concentration of the extracts employed. The effectiveness of *T. flavus* isolates applied to the seed in decreasing the Verticillium infection index in greenhouse conditions was consistent with some previous studies (Soytong and Ratanacherdchai, 2005; Kulikov *et al.*, 2006). The minor differences between the present and earlier studies could be due to differences in the way, the seed was treated with the *T. flavus* isolates, or to the interval allowed before sowing (Nagtzaam and Bollen, 1997). Other field studies have found that Verticillium wilt decreased when plants were given treatments containing *T. flavus* (Madi *et al.*, 1997; Nagtzaam and Bollen, 1997; Klosterman *et al.*, 2009).

Biological Control of Fusarium Wilt Disease Using *Talaromyces Flavus*

The causal agent of Fusarium wilt disease is *F. oxysporum* that enters the plant through the roots and pervades throughout the plant through the vascular system. A Fusarium wilt disease most expansion is in warm regions of the world and in acid and sandy soils. Disease cause will survive for a long time (over ten years) in the soil and on plant debris that this is because of the existence of resistant spores (chlamydospores).

Farhang Niya *et al*. (2015) showed that there was a possibility to biologically struggle with Fusarium wilt disease derived from *F. oxysporum* in the tomato crop with some isolates of *T. flavus*. Isolation of various *T. flavus* isolates from

tomato growing areas shows that this fungi was naturally existed in the rhizosphere of tomato. *T. flavus* occupies the rhizosphere of the crop and reducing the colony forming units of the Fusarium wilt pathogenic agents (Marois *et al*, 1984; Berg *et al*, 2005).

In the study related to Farhang Niya *et al*. (2015), the lowest disease severity percent has been related to the isolate TF-To-V-29 as an addition to the soil. The results of a research in the field of biological control of *Sclerotinia sclerotiorumin* in fields of beans and peas by the antagonistic fungus *Tricothecium roseum*, *Trichoderma virens*, *Coniothyrium minitans* and *T. flavus* showed that the efficacy of the mentioned antagonistic fungus in the treatment of addition to the soil, was more due to the increase in the establishment and their sustainability (Chang and Scott, 2000). In comparative studies between different methods of the application of the treatments affected by antagonistic microorganisms, increase in the efficiency of these microorganisms was significantly higher by the method of addition to the soil (Josh and Pan, 2007; Joshi *et al*, 2010; Patkowska and Konopinski, 2014).

Farhang Niya *et al*. (2015) showed that the treatment affected by isolate TF-To-V-29 when the increase in soil, comparing with its application with the method of adding to the soil and seed coating, was superior in term of the effectiveness of disease control. Based on this results, it can be inferred that the population of the spores of the isolate TF-To-V-29 in the method of adding to the soil was only to an extent that no barrier was created by the antagonistic activity of this isolates; but about the use of this isolate, with the method of adding to the soil and seed coating, perhaps the increase in the fungal population reduces the antagonistic activity. Previous studies have shown that the use of high concentrations of antagonistic fungus, such as materials Aspergillus and Penicillium led to the emergence of a phenomena entitled "Crowding effect". In this phenomenon, high population of fungal spores led to the production of inhibitor materials for the growth of spores (Chitarra, 2003).

For example, the population of fungal spores of *Penicillium paneum*, due to producing the 1- octen-3-OL compound (a mixture resulted from linoleic acid oxidation) with four mM concentration, will prevent the growth of spores, so that the activity of antagonistic fungi decreases. Therefore, in the present research, it has been likely that the propagator bed of the antagonist isolates acts as an incentive to increase the population of antagonistic isolates' spores and due to the mentioned phenomena, could not effect on the increase in the antagonism activity of isolates (Chitarra, 2003).

On the other hand, in the study related to Farhang Niya *et al*. (2015), the reason of the superiority of isolate TF-To-V-29 on TF-To-U-38, in term of the effectiveness of disease control, can be attributed to genetic differences and differences in their isolation areas. The results of research on the genetic diversity of different isolates of the antagonistic fungi have shown that the different intensity of enzymatic activity of the isolates was caused by genetic variation (Choudary *et al*, 2007; Siameto *et al*, 2010). In addition, the accumulation of the effective metabolites with inhibitory properties for the growth of pathogens in these isolates, was related to the favorable environmental conditions for antagonist's growth and activity (Joshi *et al*, 2010).

In the study related to Farhang Niya *et al*. (2015), in term of yield, there was no statistically significant difference between treatments in the first harvesting and the significant difference started in the second harvesting and continued to the fifth harvesting. Therefore, based on these findings, the difference in the activity of antagonist treatments in term of increase in performance, is exactly compatible to the stages of plant growth that there was a physiological readiness for crop production. In the six cutting, treatments were placed in one group in terms of average performance. It seems that in this step, the activity of the antagonistic isolate reduced due to the decrease in their population and new inoculation is

necessary for the next crop year, as well. In some studies on the biological control of soil-borne diseases of potato such as Verticillium wilt by using antagonistic fungi, the modification of these soil microorganisms in fields has been reported through applying the treatments affected by Antagonistic fungi (Lodhi, 2004; Berg *et al*, 2005; Naraghi *et al*, 2014a).

Naraghi *et al*. (2017) showed that there was apossibility of the biological control of Fusarium wilt of the cucumber caused by *F. oxysporum f. sp. cucumerinum* by different isolates of *T. harzianum* and *T. flavus*. This research was done in Varamin cucumber greenhouse, during three successive crop years. The results of the 2nd year of this research in the greenhouses of Varamin also like the first year showed that, there was a possibility of the biological control of Fusarium wilt caused by *F. oxysporum* in the greenhouse cucumber crops of some of the isolates of *T. flavus* and *T. harzianum*. In the present study, all the treatment affected by isolates of *T. flavus* (TF-Cu-V-59) by addition to the soil and TF-Cu-V-60 with seed coating and addition to the soil) and *T. harzianum* (TH-Cu-V-13 in addition to the soil and TH-Cu-V-13 with seed coating and addition to the soil), compared with the control had a significant reduction in the disease severity percent, the result was not unexpected according to the results of the research laboratory part, on reducing the growth of the pathogenic colony by the mentioned isolates (Gholi-Nyakan *et al*., 2014).

Of course, among the above treatments, despite the first year that the treatment of TH-Cu-V-13 by addition to the soil showed lower efficiency in terms of the disease control, compared to other treatments, the lowest efficiency was observed in controlling the disease in the treatment of TH-Cu-V-13, by addition to the soil along with seed coating. According to the research on the need for population threshold for the biological agent activity (Lo *et al*., 1997; Wang, 2012; Mishra *et al*., 2013), it was likely that in the first year, the needed population of the colony units was not provided for the isolate TH- Cu-V-13 by only addition to the soil, and in the second year, because of the residual of the bioformulation related to the isolate of the first year, the population of the isolate has reached the desired threshold. Uddin *et al*. (2011) showed that the occurrence of the population threshold has been possible for some of *T. harzianum* isolates to control potato seedling death, using seed and soil treatments.

In the first crop year (2013), increase yield up to 33% in the Trichoderma treatment occurred by addition to the soil and seed coating compared to only addition to the soil, while the disease severity disease in the mentioned treatment by the method of increasing the soil and seed coating also showed a 20% reduction compared to the increasing the soil. Based on the previous studies, the effect of different concentrations of various antagonist microorganisms such as *T. harzianum* and *Verticillium lecani* (10^3, 10^4, and $10^6$the colony unit per ml of suspension used to provide the seed treatments) on controlling soil borne fungal diseases in crops, such as tomato, beans and radishes showed that the difference in the colony unit even 1,000 spores in the seed treatment could cause a significant difference in the incidence of Fusarium wilt disease (Leeman *et al.*, 1996; EliRafai *et al*., 2003; and Carvalho *et al*., 2014). Thus, the inference is made that the seed treatment has been significantly important to control vascular diseases such as Fusarium wilt compared to non-vascular diseases.

In the second year, the yield of the isolate TH-Cu-V-13 by increasing the soil and seed coating was lower than the first year in terms of reducing the disease severity. Also, for the isolate due to the presence of the bio formulation residual related to the last year, the population of the colony units of the isolate exceeded the threshold and according to the previous studies (Chitarra, 2003) the phenomenon of removing fungal colony units occurred by the fungus and consequently its antagonist activity has been reduced.

On the other hand, the results of the present study showed that the capability of different antagonistic fungal species (*T. flavus* and *T. harzianum*) and even the isolates related to a species (TF-Cu-V-59 and TF-Cu-V- 60) were

different in terms of the yield increase. In this field, the different efficiency of endophytic fungus isolates *Gibberella fujikuroi* has been showing on strengthening vegetative traits such as the stem length in some ofdicotyledonous plants including *Vitex rotundifolia*, *soldanella calystegia* and *Polygonum convolvulus* (Khan et al., 2012). In another study, also Contreras-Cornejo *et al*. (2009) showed that the species *Trichodermaatroviride* and *Trichodermavirens* due to the difference in the amount and activity of root growth stimulating hormones (auxin and its derivatives), had different strengthening properties for the plant growth.

Also, the results of the 2^{nd} study in Varamin greenhouse showed that for both antagonistic fungi *T. flavus* and *T. harzianum,* only adding to the soil has been more effective in increasing the yield, in terms of efficiency compared to addition to the soil and seed coating. According to the results of Chitarra (2003) study, reducing the efficiency of some antagonist isolates in the biological control, and thus, reduce the plant yield can be attributed to the reduced population.

Thus, the use of high concentrations of spores of antagonist fungi such as *Aspergillus* and *Penicillium*genus cause the above-mentioned phenomenon (Crowding effect).

Biological Control of Seedling Damping-off Disease Using *Talaromyces Flavus*

Seedling damping-off is a soil-borne disease that can attack almost all young vegetable seedlings. Home gardeners often first notice that something is wrong when the very young seedlings have a constriction around the base of the stem, some of the seedlings have fallen over or there are small flying gnats around the base of the seedlings. Damping off is caused by soil fungi and the right conditions to help them flourish. If not taken care of, losses can be severe and result in the majority of a flat of carefully planted seedlings dying in 24 to 48 hours. Damping-off can happen before seedlings emerge from the soil. With this type of damping-off, fungi infect seeds as they germinate. As the infection progresses, seeds rot and do not germinate, leading many home gardeners to think that the seed quality is poor. Another result of seedling infected damping-off is poor or weaker seedlings that become apparent days or weeks later.

The most common cause of damping off is from soil-borne fungi from three groups – Pythium, Rhizoctonia, and Fusarium. *Pythium* thrives in cool, over-wet and poorly-drained soils and is often the result of over-watering and not maintaining a warm enough soil temperature for the seeds to germinate. Its symptoms are a damp, odorless rot in the root, causing it to be slimy. It may run up the lower portion of the stem and cause it to be black and slimy. Pythium can survive in soil for several years. *Rhizoctonia* is present in all natural soils, coming to life when a soil is over-wet and hot. This is the most common occurrence of damping off, as the seedling will have the classic constriction on the stem right around where it touches the soil. *Fusarium* thrives in acidic soils that are poorly fertilized and can remain inactive for long periods of time – years. Fusarium infects the seeds, causing many of them to fail to germinate and creating the 'wire-stem' appearance in those that do survive. There are also seed-borne bacterial and fungal pathogens that can decimate seedlings. These are most often seen in seeds obtained from seed swaps or gardening clubs with poor sanitation and handling techniques in processing and packing seeds. If not recognized and corrected, seed-borne pathogens will continue to infect future generations of seed that is saved and distributed.

Naraghi *et al*. (2014b) showed that it might be possible to promote health and growth of sugar beet using Talaromyces and Trichoderma fungal antagonists. These fungal antagonists were capable of both disease suppression and promotion of growth and yield of sugar beet in the greenhouse as well as field conditions. *Trichoderma* and *Talaromyces* have previously been used in the biological control of several plant diseases, including cotton seedling damping-off,

cucumber wilt, potato wilt and tomato wilt diseases (Howell, 2002; Naraghi *et al*., 2010a, b and c). Seedling damping-off which is one of the most important diseases of sugar beet around the world has recently been controlled by the application of different microbial antagonists, including fungi and bacteria (Shahiri Tabarestani, 2005; Shahraki *et al*., 2008; Jorjani *et al*., 2011).

In the greenhouse experiment related to Naraghi *et al*. (2014c), the treatments affected by isolates of *T. flavus* had significantly greater numbers of healthy seedlings in the case of soil artificially inoculation with Rhizoctonia, or a combination of pathogenic factors compared to the inoculation with Fusarium, while a significant increase was observed in the number of healthy seedlings in soil artificially inoculated with Fusarium compared to the soil inoculated with Rhizoctonia or combination of pathogenic factors for treatments affected by isolates of *T. harzianum*. Therefore, it can be concluded that the effect of *T. flavus* on the reduction of sugar beet damping-off disease caused by *R. solani* or combination of pathogenic factors was higher than the case in which Fusarium was the only cause of this disease. However, the maximum effect of *T. harzianum* on the reduction of sugar beet damping-off disease occurred when its only cause was Fusarium. In this regard, the results of the previous study by Nicoletti *et al*. (2009) also indicated that the effect of *T. flavus* on the inhibition of the growth *R. solani* was higher than other fungal pathogens of seedling damping off.

Furthermore, the results of the field studies related to Naraghi *et al*. (2014c) indicated that the treatment, affected by isolate of *T. flavus* (T. F. K.3) had the maximum yield compared with other treatments and it had no significant difference to the treatment affected by isolate *T. harzianum* (T. H. K.1). According to the results of previous studies by Moayedi and Mostowfizadeh-Ghalamfarsa (2010) in the sugar beet field, the population of soil microorganisms after application of fungal antagonist, isolates of *T. harzianum* and *T. flavus* during the first crop year of this research led to the breeding population of these isolates especially *T. flavus*, so that they could maintain the effects of metabolites until the end of the growth period. According to the greenhouse and field experiments in the above-mentioned study, the difference between the antagonistic isolates of *T. flavus* or *T. harzianum* in terms of their capability to reduce the seedling damping-off disease could be caused by a wide range of activities and mechanisms, including the production and secretion of different metabolites by these isolates which may be due to their genetic variation (Madi *et al.*, 1992).

Biological Control of *Polymyxa Betae*, Vector Rhizomania Disease Using *Talaromyces Flavus*

Rhizomania is a plant disease of the Chenopodiaceae family caused by the development of a virus called BNYVV (Beet Necrotic Yellow Vein Virus), which is introduced and transmitted by *Polymyxa betae*. Rhizomania is a virus disease which may cause heavy losses in yield and quality.

Naraghi *et al*. (2014b) showed that it might be possible to decrease rhizomania disease by reducing its vector population, using *Talaromyces* and *Trichoderma* fungal antagonists. In this study, these fungal antagonists were capable of both disease suppression and promotion of sugar beet growth factors, in greenhouse conditions. Among the different methods for application of the above-mentioned antagonists, the soil treatment method was the most efficient in reducing the *P. betae* population. Under greenhouse conditions, the incidence of the disease in the plants sown in the infested soil revealed that the soil had a potential pathogenicity for the sugar beet. Results of a previous study on the dispersion of *P. betae* in Isfahan, West Azerbaijan, and the Khorasan-e-Razavi provinces of Iran, showed the presence of 164–480 cystosori in one gram of root in sugar beet fields infested with rhizomania. This amount was an indicator of the high probability of the disease occurrence (Jalali *et al*., 2009).

So far, no study has been done on biocontrol of the vector of sugar beet rhizomania in Iran. However, results of the above-mentioned study about the reduced resting structure of the disease vector in treatments affected by *T. flavus* and *T. harzianum* agree with the results of the few previous studies in other countries using antagonistic fungi and bacteria. For example, in a study on the biocontrol of rhizomania using antagonistic isolates of *T. harzianum*, D'Ambra and Mutto (1986) showed that these isolates could parasitise and decompose the resting structures of the disease vector. In a similar study on the antagonistic effects of *Pseudomonas putida* on the fungal vector, the bacterium biotypes A and B reduced the disease vector populations by 23% and 75%, respectively (Aksoy and Yilmazz 2008).

Moreover, the results of the present study in the treatments affected by antagonistic isolates with different methods of application, showed that the resting structure of the vector was reduced by all antagonistic isolates used as a soil treatment. In the previous studies which used antagonistic bacteria and fungi only in the form of spore suspension, the results were somehow different compared with the results of the study related to Naraghi *et al*. (2014b). It could be argued that the high effectiveness of antagonistic fungi used as a soil treatment compared to the seed treatment is due to the higher populations in the inocula added to the soil (Choi 2003; Adandonon *et al*. 2006). In the study related to Naraghi *et al*. (2014b), the soil treatment method was more effective than the combination of the seed and soil application methods. This might be due to a "crowding effect". The phenomenon that was described in the before sections.

Based on a greenhouse experiment, Jakubikova *et al*. (2006) refereed certain *T. harzianum* isolates with an inhibition capability of 21–68%, for proliferation of the pathogenic virus (BNYVV), as the biological factors effective in the control of rhizomania in sugar beet fields. The results of the previous studies have also shown that biological control agents are effective against damping-off, root rot, and wilt diseases of ornamental plants, vegetables and cereals, caused by *Rhizoctonia, Pythium*, *Phytophthora, Sclerotium*, *Fusarium* and some other fungi (Whipps 2001; Asef *et al*. 2008; Naraghi *et al*. 2010a, b, c; Godhani 2011; Ojaghian 2011).

Naraghi *et al*. (2014b) also revealed that maximum reduced cystosori populations of the fungal vector and a maximum increased weight of sugar beet roots were respectively obtained in TF-Su-M-2 and TH-Su-M-1 treatments, using the soil application method. Therefore, these treatments may be used in field studies. Based on the results of the study related to Naraghi *et al*. (2014b), recent biological formulations could be used in the field. In a field study, *T. harzianum* has been applied for soil and seed treatments as 2×10^{12} spores/ha (2 kg/ha) and 8 g/kg seed, respectively (Godhani 2011).

BioControl Agent *Talaromyces Flavus* Stimulates the Growth of Some Important Crops

Narathi *et al*. (2012a and b) showed that it might be possible to promote growth in some plant characteristics by different *T. flavus* isolates. The results of these studies reveal the potential of *T. flavus* for increasing plant growth in cotton and potato. The type of growth promotion may be similar to that produced by the addition of *Trichoderma* spp. which has been found to enhance the growth of various plants (Chang *et al*., 1986; Paulitz *et al*., 1986; Windham *et al*., 1986; Baker, 1988; Kleifeld and Chet 1992; Ousley *et al*., 1994a and b; Phuwiwat and Soytong, 1999; Harman, 2000; Contreras-Cornejo *et al*., 2009; Hajieghrari, 2010; Masunaka *et al*., 2011).

The increased plant growth induced by *Trichoderma* spp. has been shown to be dependent on many factors such as plant species, the strain of Trichoderma, the form of inoculum, the concentration of inoculum, and the soil environmental conditions (Paulitz *et al*., 1986; Baker, 1988; Kleifeld and Chet, 1992; Ousley *et al*., 1994a and b). In the studies related to Naraghi *et al*. (2012a and b), it was shown that *T. flavus* had a significant effect on the growth of cotton,

potato, tomato and cucumber by increasing root length, crown length, plant height, plant fresh weight, and plant dry weight. In this regard, there are previous reports that *T. flavus* and *Penicillium notatum* may enhance plant growth directly (Phuwiwat and Soytong 2001; Bewley *et al*., 2006).

Phuwiwat and Soytong (2001) showed that *P. notatum* isolated from rhizospheric soil gave higher plant yields of treated Chinese mustard compared to non-treated plants. The Chinese mustard grown in sterilized planting medium mixed with *P. notatum* yielded the highest plant growth. Plant height, root length, root diameter, fresh and dry weights of shoot and root, and the total plant dry weight increased gradually as the inoculum of *P. notatum* was applied. Penicillium species (including the teleomorphic states of Talaromyces and Eupenicillium) are considered a key group of soil microflora involved in P cycling (Whitelaw, 2000). This activity is generally attributable to the production of organic acids that can directly dissolve P precipitates, or chelate P-precipitating cations with the concomitant release of P into solution (Kucey *et al*., 1989; Gadd, 1999). This could be an important reason for the increase in growth in cotton and potato observed in the present study.

However, many studies have shown that *T. flavus* is capable of controlling some important soil-borne pathogens such as *Verticillium dahliae*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, and *S. rolfsii* in several crops, including cotton, potato, tomato, eggplant, and bean (Dutta, 1981; Madi *et al*., 1997; Tjamos and Fravel 1997; Menendez and Godeas, 1998; Naraghi *et al*., 2006). Naraghi *et al*. (2010a, b, c) reported that *T. flavus* could reduce Verticillium wilt disease caused by *V. alboatrum* in potato, tomato, and greenhouse cucumber. Despite the presence of *T. flavus* in cotton and potato fields of Iran, its population density is not enough to mediate soil nutritional processing. It should be added to the soil as inoculum until it gets active for decomposition, nutrient mobilization, mineralization, and storage and release of nutrients and water (Pandya and Saraf, 2010).

There is overwhelming evidence in the literature indicating that plant growth-promoting fungi (PGPF) can be a true success story in sustainable agriculture (Sudha *et al*., 2011). In fact, through their numerous direct and indirect modes of action, PGPF can allow a significant reduction in the use of pesticides and chemical fertilizers. The beneficial effects of PGPF, that is, biological control of diseases and pests, promotion of plant growth, increase in crop yield, and improvement in crop quality, can take place simultaneously or sequentially. Plant age and the soil’s chemical, physical, and biological properties will greatly influence the outcome of PGPF inoculation (Hyakumachi and Kubota, 2004; Siddiqui *et al*., 2008). Presently, the absence of a universal magic PGPF bio inoculant formulation for each important field crop simply reflects the complexity of the interactions and of the molecular signal exchanges taking place in the soil-plant-organisms ecosystems (Arora, 2004).

It would be interesting to test *T. flavus* for biological control of important plant pathogens. Further investigations are needed to determine the potential of this fungus in promoting the growth of different crop plants. The effective strains would need to be selected and the suitable concentrations for plant growth promotion must be investigated. Moreover, the soil environmental conditions that are suitable for promoting plant growth also have to be studied. The results of the previous studies are promising and may be used in the biological fertilization of cotton and potato in the field. Because the tested fungal isolates have shown both antagonistic and plant-growth-promoting properties, their successful application in the field may result in the reduction in the use of chemicals in agriculture and protection of the agricultural environment and biological resources which are very important factors for a sustainable agricultural system.

Development of Bio Formulations Containing *Talaromyces Flavus*

Naraghi *et al*. (2007) studied for the growth ability of *T. flavus* on different plant material residues for biological control of cotton wilt caused by *Verticillium dahliae*. In this study, the antagonistic fungus, *T. flavus* was grown on several natural culture media such as straw, wheat bran mixed with straw, rice bran, rice husk, corn-cob and fertilizer mixed with soil worm. Sporulation of *T. flavus* was then compared on these culture media three weeks after incubation. Results indicated that maximum and minimum sporulation of *T. flavus* were 5.4×10^{9} and 1.87×10^{9} spore per gram on cob and fertilizer mixed with soil worm culture media respectively. In the next step of this research, the sporulation process of *T. flavus* on different natural media was studied monthly up to nine months after incubation. Results showed that the mean of the spore number per gram of inoculum for six natural media, increased from three weeks to four months after incubation. This mean was constant for two months and after this time decreased. Results also showed that maximum and minimum sporulation of *T. flavus* were obtained on cob and fertilizer mixed with soil worm culture media respectively. The growth, stability of *T. flavus* on natural media was investigated up to eighteen months after incubation with an interval of three months.

The results of this experiment, showed that the percent growth, stability of this fungus on all media increased from three to six months after incubation and then decreased gradually. Maximum and minimum percent growth, stability in sixth month were 100, 100, 100 and 20 % on corn-cob, rice bran, wheat bran mixed straw and fertilizer mixed with soil worm culture media respectively. The antagonistic ability of different inoculums of *T. flavus* prepared for natural media was evaluated in greenhouse conditions by calculating the mean of infection index in treatments contained inoculums of *T. flavus* and *V. dahliae* compared to treatment contained *V. dahliae* only. Results of this study, showed that maximum antagonistic ability or minimum infection index (2.77) was caused by treatment contained inoculum of *T. flavus* prepared on rice bran compared to that of treatment contained *V. dahliae* only which was calculated as 11.51.

The results of the other studies (Naraghi *et al*., 2010a, b and c; Kakvan *et al*., 2013) indicated that mixing fungal antagonists (*T. flavus* or *T. harzianum*) with organic and inorganic carriers preserved their antagonistic potential for controlling some important plant diseases such as seedling damping-off, Verticillium wilt and Fusarium wilt.

Effectiveness of the Chemical Stabilizers used in *Talaromyces Flavus* Bio Formulations on Biological Control of Some Important Plant Diseases

In the research related to Bahramian *et al*. (2016), the application of *T. flavus* bioformulations including several chemical stabilizers such as sodium nitrate, carboxymethyl cellulose, D-cycloserine and magnesium sulfate led to significant decrease the incidence of some important soil-borne fungal diseases such as seedling damping-off in tomato and greenhouse cucumber.

The results of the other research also showed that some chemical compounds, including sodium nitrate, potassium phosphate, magnesium sulfate, L-asparagine, L-sorbose caused the growth inhibition of the several soil-borne fungal pathogenic agents such as Verticillium and Fusarium (Ausher *et al*., 1975; Christen, 1982; Hadar and Katan, 1989; Veverka *et al*., 2007). Bahramian *et al*. (2016) showed that *T. flavus* bioformulation containing sodium nitrate was effective in controlling tomato and greenhouse cucumber seedling damping-off.

On the other hand, an osmotic stabilizer such as sodium nitrate was reported as the stabilizing compound for the chitinase enzyme (Gavanji *et al*. 2013; Patil and Jadhav, 2015). Such a compound could therefore play an important role in

the maintenance of the metabolite related to the mycoparasitism mechanism of *T. flavus* which is a chitinase enzyme (Inbar and Chet, 1995). In the research related to Bahramian *et al*. (2016), no differences were observed in tomatoes and greenhouse cucumbers in terms of the impact of two diverse *T. flavus* bioformulations with carboxymethyl cellulose and D-cycloserine stabilizers to control the diseases under study. The results of this research showed that bioformulations containing *T. flavus* isolates related to tomato and greenhouse cucumber simultaneously with sodium nitrate stabilizers and D-cycloserine exhibited desirable efficiency in controlling the Verticillium and Fusarium wilts.

No precise information is available on the application of additive substances to biological compounds in order to increase their stability. But there are considerable reports that indicate the efficiency and stability of biological compounds are the most important factors in marketing and commercialization of these products (Mukhopadhyay and Maiti, 2009; Kaewchai *et al*., 2009; Ghaderi-Daneshmand *et al*., 2012).

CONCLUSIONS

The overall results of the previous studies that have been mentioned in the present review article show that it may be possible to manage some important plant diseases on several major crops using *Talaromyces flavus,* a novel bio fungicide. These results indicate that this antagonistic fungus posses growth promotion ability and is capable of promoting growth characteristics of cotton, potato, sugar beet, tomato and greenhouse cucumber.

Seedling damping-off, Verticillium wilt and Fusarium wilt are the important diseases of cotton, sugar beet, tomato, potato and greenhouse cucumber that cause serious losses in these crops every year. Application of chemical fungicides is not effective in controlling these diseases. The use of resistant varieties is efficient, but it's time-consuming and costly as well. Biological control is one of the most suitable strategy for controlling this disease. It is environmentally safe and is not very costly.

Results of the previous studies showed that *T. flavus* isolates caused significant decrease in disease index and a significant increase of growth characteristics (root length, crown length, plant height, fresh weight and dry weight) compared to infected control. Results also showed that there was the significant decrease in disease index and the significant increase in growth characteristics in some treatments contained *T. flavus* compared to control. The overall results of the reviewed studies indicate that use of *T. flavus* isolates could be a viable strategy for controlling some important plant diseases in cotton, greenhouse cucumber, potato, sugar beet and tomato. According to the results of the previous studies, different *T. flavus* isolates that were obtained from different regions and were diverse in their genetics, caused a significant decrease in disease and increase in growth characteristics of above-mentioned crops. Results of these studies may be used in integrated pest management of this disease and result in an increase of production, decrease in the application of chemical fungicides, protection of the environment and biological resources and approaches a sustainable agriculture system.

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